Microbial Cells Harboring a Mitochondrial Gene Are Capable of CO₂ Capture

Yanchao Zhou^{a,*}, Lan Ouyang^{a,*}, Xiao Yi^{a,*}, Tao Gan^{a,b,*}, Jinhuan Qi^{a,*}, Yulin Wan^{a,*}, Yuchuan Wang^c, Shanshan An^a, Yunfan Shi^a, Wei Yang^a, Wenze Chen^a, Zhiyao Luo^a, Jing Li^{a,d}, Jun Luo^e, Xiren Nuertai^a, Xiang Zhu^a, Fan Yang^a, Beibei Zhao^a, Weiwei Zhang^a, Zi-Wei Ye^f, Xiaoxiao Zhang^a, Shaoping Weng^a, Qiuyun Liu^a, Weiguo Cao^{a,d} & Jianguo He^a

^aState Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Improved Variety Reproduction in Aquatic Economic Animals, Biomedical Center, Lab of Microbial Metabolic Engineering and Synthetic Biology, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China.

^bDepartment of Biotechnology, School of Basic Medicine, Gannan Medical University, Ganzhou 34100, China.

^cSchool of Chemistry, Sun Yat-sen University, Guangzhou 510275, China.

^dDepartment of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA.

^eDepartment of Mathematical Sciences, Clemson University, Clemson, SC 29634, USA.

^fDepartment of Microbiology, The University of Hong Kong, China.

*These authors contributed equally to this work.

Correspondence and requests for materials should be addressed to Q.L. (lsslqy@mail.sysu.edu.cn), W.C. (wgc@clemson.edu) or to J.H. (lsshjg@mail.sysu.edu.cn)

ABSTRACT Global warming is escalating with increased temperatures reported worldwide. Given the enormous land mass on the planet, biological capture of CO₂ remains a viable approach to mitigate the crisis as it is economical and easy to implement. In this study, a gene capable of CO₂ capture was identified via selection in minimal media. This mitochondrial gene named as *OG1* encodes the OK/SW-CL.16 protein and shares homology with cytochrome oxidase subunit III of various species and PII uridylyl-transferase from *Loktanella vestfoldensis* SKA53. CO₂ capture experiments indicate that $\delta^{13}\text{C}$ was substantially higher in the cells harboring the gene *OG1* than the control in the nutrition-poor media. This study suggests that CO₂ capture using engineered microorganisms in barren land can be exploited to address the soaring CO₂ level in the atmosphere, opening up vast land resources to cope with global warming.

IMPORTANCE Global warming crisis is deteriorating with increased CO₂ levels in the atmosphere each year. Action must be taken before catastrophic consequences occur in the not-so-distant future. Biological capture of CO₂ is a feasible approach to alleviate the current crisis. We have identified a mitochondrial gene which demonstrated CO₂ utilization capability. Data presented in this study suggest that CO₂ capture using engineered microorganisms can be harnessed to address the ever-rising CO₂ level in the atmosphere.

KEYWORDS CO₂ capture, OK/SW-CL.16, $\delta^{13}\text{C}$, cytochrome oxidase subunit III

It is widely believed that the combustion of fossil fuel contributes to the rising CO₂ level in the atmosphere, by a surge of estimated 100 ppm since its pre-industrial level¹. Global temperature has risen by more than half a degree in the past 50 years as a result of greenhouse gas emissions. Current technologies for carbon capture and storage and chemical fixation of CO₂ are varied². The amount of CO₂ captured relative to the magnitude of CO₂ emitted and energy required are two major concerns. From this point of view, biological CO₂ capture is a viable approach with minimal energy demand. Here we describe the cloning of a mitochondrial gene which is able to capture CO₂, and the engineered microbes showed great potential in addressing the global warming issues.

Results

To search for genes that can capture CO₂, we constructed and screened a random DNA library. The effort has ended with a sequence from an unknown DNA library, which shares homology with the genome of *Homo sapiens* haplogroup H63 mitochondrion (Supplementary Fig. S1) and gave rise to *Escherichia coli* colonies in nitrogen free media. More importantly, these colonies were later shown to be able to grow on carbon deficient media. Supplement in rich media Luria Broth with vitamin C was essential for the successful sequencing of the positive clone pOG, suggesting that the OG-encoded protein might catalyzes a stressful oxidative reaction. The protein encoded in the subclone pOG1 is identical to a known protein OK/SW-CL.16³, which shares homology with PII uridylyl-transferase from *L. vestfoldensis* SKA53 and cytochrome oxidase subunit III from *Macaca mulatta* (Supplementary Fig. S2). The OK/SW-CL.16 protein harbors a class I PxxP motif, a predicted actinin-interacting region, and a RIM1-like sequence³. Previous study has shown that OK/SW-CL.16 acted as a binding partner for actinin-4³.

Yeast extract was included in carbon-free media to allow proton traffic and attenuate osmotic pressure since its peptide and protein constituency are rich in charged amino acids and hydrogen bond donors and acceptors (Supplementary Fig.

81 S3 and S4). Without supplement of minimal amount of proteins and peptides, pOG1
82 grew very poorly in carbon free nutrition-poor liquid media (Supplementary Fig. S3).

83 The commercially available ^{13}C -Urea Breath Test was based on the release of
84 CO_2 by urease^{4,5}. It was adapted in this study to investigate the potential capacity of
85 CO_2 capture using our clones. Isotopic measurements at the end of carbon fixing
86 experiments (Fig. 1; Supplementary Tables S1-S8) indicate that $\delta^{13}\text{C}$ was substantially
87 different between the clones harboring OG1 gene and the control clone in both *E. coli*
88 and *Saccharomyces cerevisiae* (Fig. 1A and 1D), suggesting that the clones harboring
89 OG1 gene may have higher assimilation rate toward CO_2 . Since microbial cells were
90 submerged in 50 ml liquid media without shaking, it suggests that engineered
91 microbial cells might be able to capture CO_2 under surface soil. ATP supplement
92 diminished the CO_2 fixing activity of the pOG1 clone (Fig. 1A). Although a clone
93 pOGDR1 obtained from directed evolution had significantly higher $\delta^{13}\text{C}$ than pOG1
94 clone at pH 8.0, its $\delta^{13}\text{C}$ value was lower than that of pOG1 clone at pH 7.0 (Fig. 1G
95 and 1J; Table 1), suggesting that proton traffic may be integral in CO_2 capture.
96 Treatments with the carbonic anhydrase inhibitor acetazolamide generated no
97 difference in $\delta^{13}\text{C}$ patterns between pOG1 groups and pSK- groups ($p=0.567$; Fig. 2,
98 S9 and S10), suggesting that spontaneous hydration of CO_2 was present. Alternatively,
99 the *E. coli* carbonic anhydrase may be insensitive to acetazolamide. *OG1* was highly
100 expressed in engineered *E. coli* host cells, which was determined via Realtime
101 fluorescent quantitative PCR (Fig. 3; Supplementary Tables S11 and S12).

102

103 Discussion

104 The subclone pOG1, identical to a known protein OK/SW-CL.16, shares homology
105 with numerous proteins including Cytochrome c oxidase subunit III. It is a constituent
106 of the multi-subunit cytochrome c oxidase, and part of the respiratory chain
107 of mitochondria and aerobic bacteria^{6,7}. It catalyzes the following reaction:

109 The utilization of the four protons above might be tied to the hydration of CO₂
110 below, which leads to dissociation of protons and generation of bicarbonic acid.

112 Bicarbonic acid could be assimilated as carbon source⁸, and accumulated protons
113 could be utilized by OG1 protein in the aforementioned first reaction coupled with
114 second reaction via proton traffic to produce water molecules without generating
115 excessive osmotic pressure.

116 The pOG1 clone was initially isolated in nitrogen free media. Although acetylene
117 reduction assays indicated no obvious nitrogen fixation activity with pOG1 clone,
118 weak activity may arise endogenously in the host upon the potential regulation by the
119 OK/SW-CL.16 protein that shares homology with PII uridylyl-transferase, which acts
120 as regulator of the cellular nitrogen and carbon status in prokaryotes and plants⁹, such
121 as NifA activity regulation and ammonium-dependent post-translational regulation of
122 nitrogenase in *H. seropedicae*¹⁰.

124 In response to nitrogen limitation, the uridylylated PII protein promotes
125 deadenylation of glutamine synthetase (GlnA), consequently activates the enzyme
126 and stimulates NtrC-dependent promoters¹¹.

127 The P108T substitution in pOGDR1 had profound impact on carbon fixing
128 outcome in media with different pH values. The σ_{22} elements of the ¹³C chemical shift
129 anisotropy for the deprotonated carboxyl groups indicate stronger hydrogen bonding
130 of carbonyl oxygen in threonine than that in either proline or serine¹². The
131 electron-withdrawing inductive effect of the hydroxyl oxygen atom renders the
132 hydroxyl oxygen in β -branched threonine less chemically active than its counterpart
133 in serine, for instance, in the formation of hydrogen bonds. These properties will
134 impact enzyme catalysis by the protein of OK/SW-CL.16 variant as a whole. The
135 potentially non-casual connection between respiration chain or ATP's effect and CO₂
136 usage suggests that the mitochondrial genome may harbor other genes for CO₂ capture.
137 Another potential application of the *OGI* gene would be serving as a selective marker

138 by growing on carbon deficient minimal media and replacing antibiotic selective
139 markers in constructed plasmid.

140 Given the minimal nutrition it requires and potential regulation by soil ions and
141 other substances on proton traffic, the pOG1 clone could be dispersed in barren lands
142 to capture large amount of atmospheric CO₂ to cope with escalating global warming
143 crisis.

144

145 **Methods**

146 **Cloning and Subcloning of the gene capable of CO₂ capture.** Chimeric DNA
147 fragments encoding *S. cerevisiae* α -factor secretion signal and random peptides were
148 generated from PCR amplifications of pPICZ α A (Invitrogen, Carlsbad, CA, USA)
149 linearized with BamH I. The two primers were: (1)
150 GCGGATCCAAAAATGAGATTTCCTTCAA;(2)CGTCTAGATCAACCN(T/C)(G/
151 C/T)N(T/C)(G/C/T)ACAN(T/C)(G/C/T)N(T/C)
152 (G/C/T)ACCAGATGGN(T/C)(G/C/T)N(T/C)(G/C/T)
153 ACAN(T/C)(G/C/T)N(T/C)(G/C/T)AGATCTTTTCTCGAGAGATACCC. PCR
154 reactions were performed using *Pfu* and *Taq* DNA polymerases (Takara, Dalian,
155 China) in the presence of 2.5% pyrrolidone (Tokyo Kasei Kogyo Co. Ltd., Chuo-ku,
156 Japan). After a preheating step at 94 °C for 2 min, PCR amplification was performed
157 with 40 cycles of incubation at 95 °C for 20 s, 20 °C for 60 s and 72 °C for 30 s, and a
158 final extension at 72 °C for 5 mins. PCR product was ethanol precipitated and dried.
159 Double digestion with Xba I and BamH I was subsequently conducted for 5 h.
160 pYES2/CT-ADH2 Vector¹³ was triple digested with *Bam*H I, *Xho* I and *Xba* I¹⁴, and
161 then ligated with double digested PCR product at 6 °C overnight. Ligation mixture
162 was electroporated to *E. coli* ORIGAMI cells (Novagen, Madison, WI, USA) at 12.5
163 kV/cm, followed by plating to nitrogen fixation media (M9 media with NH₄Cl
164 removed, and supplemented with 1% sucrose as carbon source)¹⁵. One colony
165 appeared after growing at 30 °C for 3 days. The positive clone pOG was unable to

grow after 3-month storage at the -20 °C freezers, and subsequently revived by 1-min UV irradiation in a laminar hood followed by plating to nitrogen fixation media described above. It was then successfully sequenced only after adding 5 mg/L Vitamin C to LB for cell propagation. pOG was subsequently amplified with Taq DNA polymerase with the following 2 primers: F0: GACGGTATCGATAAGCTTGATATCGAATTCATGACCCCTAACAGGGG C; PB2: ACTAGTGGATCCCCCGGGCTGCAGCTATGGTGAGCTCAGGTGATTGATAC TCCTGATGC. After a preheating step at 94 °C for 3 mins, PCR amplification was performed with 35 cycles of incubation at 95 °C for 20 s, 42 °C for 45 s and 72 °C for 90 s, and a final extension at 72 °C for 5 mins. The PCR product was purified with the QIAquick gel extraction kit (QIAGEN, GmbH, Hilden, Germany) per manufacture's instruction. Both PCR amplicons and SK- vector were double digested with *EcoR* I and *BamH* I at 37 °C for 4 h, which were subsequently purified with the QiaQuick kit. Ligation was carried out at 16 °C overnight before electroporating into *E. coli* MG1655 cells. Positive clone pOG1 was identified after plasmid sequencing.

¹³C-CO₂ assimilation assays. pSK- clone and pOG1 clone in MG1655, and MG1655 cells were inoculated to LB respectively, and propagated at 37 °C at 120 rpm for 19 h. The cell pellets were washed once with sterile water and resuspended in sterile water. 4 OD (600 nm) of cells were inoculated to 50 ml CO₂ capture media (0.02% YNB (Yeast Nitrogen Base, nitrogen free, Amresco, Inc., Solon, Ohio), 0.01 % ammonia sulfate, 0.04% yeast extract, pH 8.0 adjusted with NaOH) with ampicillin (60 mg/L) or no ampicillin for MG1655. Six layers of cheese cloth were used as caps to allow CO₂ in. Vacuum desiccator was adapted for use with atmosphere inside. CO₂ was released by hydrolysis of 6 mg urea-¹³C (99 atom %, Sigma Aldrich, Missouri, USA) by 0.2 mg of urease from Jack Bean (Tokyo Chemical Industry Co. Ltd., Chuo-ku, Tokyo, Japan) in PBS (pH 7.4) in a total volume of 150 µl. The apparatus was sealed with Vaseline, and cells were cultured inside for 96 h at 30 °C. *E. coli* MG1655 cells was cultured outside of the device with ordinary airtight cap at the same temperature

195 and with the same incubation time. Subsequently absorbance at 600 nm was measured.
 196 Bacteria were centrifuged for 10 min at 7084 x g, and the pellets were washed with
 197 20-30 ml of sterile water and mixed well followed by centrifugation for 5 min at 7084
 198 x g. Pellets were transferred to 1.5 ml Eppendorf tubes followed by drying at 60 °C
 199 for 1 h. For *S. cerevisiae*: Sample PYES2OG1 clone and control pYES2/CT/ α -Factor
 200 clone in INVSc1 were divided into two groups with five replicates each. 5 OD (600
 201 nm) of cells were inoculated to 50 ml CO₂ capture media (0.02% YNB, 0.01 %
 202 ammonia sulfate, 0.04% yeast extract, pH 8.0 adjusted with NaOH, adding 1x amino
 203 acids concentrate of final concentration before use) with ampicillin (100 mg/L). 100 x
 204 amino acids concentrate (Ura⁻) contained 5 g Adenine, 5 g leucine, 5 g tryptophan and
 205 2.5 g histidine per liter. CO₂ was released by hydrolysis of 6 mg urea-¹³C (99 atom %,
 206 Sigma Aldrich, Missouri, USA) by 0.2 mg of urease from Jack Bean (Tokyo Chemical
 207 Industry Co. Ltd., Chuo-ku, Tokyo, Japan) in 10 mM PBS (pH 7.4) in a total volume
 208 of 150 μ l. The rest is as described above.

209

210 **Determination of stable carbon and nitrogen isotope ratio.** Experiments were
 211 carried out in the Environmental Stable Isotope Lab, Chinese Academy of
 212 Agricultural Sciences¹⁶. 2-4 mg of sample was weighed in a tin foil cup, and was
 213 subsequently introduced into the elemental analyzer (Vario PYRO cube, Elementar,
 214 Germany) through an automatic sampler, where the sample was burned and reduced
 215 into pure CO₂ and N₂. The gases were further diluted in a dilution apparatus, and
 216 measured in the stable isotope ratio mass spectrometer (IsoPrime100, Isoprime,
 217 England). The detailed running parameters were as follows: Elemental Analyzer:
 218 Burner temperature: 1020 °C; Reducing furnace temperature: 650 °C; He carrier gas
 219 flow rate: 230 mL/min. Dilution Apparatus: He dilution pressure: 4 bar; CO₂
 220 reference gas pressure: 8 psi; N₂ reference gas pressure: 8 psi. Mass Spectrometer:
 221 CO₂ reference gas was calibrated *via* USGS24 ($\delta^{13}\text{C}_{\text{PDB}} = -16\text{‰}$) through two point
 222 corrections, and the results were corrected by USGS24 and IAEA600 ($\delta^{13}\text{C}_{\text{PDB}} =$

223 -27.5‰). N₂ reference gas was calibrated *via* IAEA N1 ($\delta^{15}\text{N}_{\text{air}} = 0.4\text{‰}$), and the
224 results were corrected by IAEA N1 and USGS43 ($\delta^{14}\text{N}_{\text{air}} = 8.44\text{‰}$).

225

226 **Liquid culture in carbon fixing media with or without yeast extract.** Carbon
227 fixing medium A contained 0.2 g/L YNB, 0.1 g/L ammonia sulfate, 0.4 g/L yeast
228 extract, with sodium hydroxide adjusting pH equal to 8.0, supplemented with 100
229 µg/ml ampicillin. Medium B was as above but free of yeast extract. Two samples
230 designated pOG1 and SK- in *E. coli* MG1655 were propagated overnight in LB
231 medium with 100 µg/ml ampicillin at 37 °C and aerated by shaking at 220 rpm.
232 Cultures were centrifuged for 10 min at 6000 rpm and washed with sterile ultrapure
233 water twice after reaching logarithmic phase. The washed bacteria with OD_{600nm} of
234 0.2 were inoculated into carbon fixing medium with 100 µg/ml ampicillin. Each group
235 had four replicates. OD_{600nm} was determined after incubation at 30 °C for 3 days
236 (Supplementary Fig. S3).

237

238 **pH tolerance test.** Carbon fixing medium contained 0.2 g/L YNB, 0.1 g/L ammonia
239 sulfate, 0.4 g/L yeast extract, with sodium hydroxide adjusting pH equal to 5.0, 6.0,
240 7.0, 8.0 and 9.0 respectively. Bacterial cultures were propagated overnight in LB
241 medium with 100 µg/ml ampicillin at 37 °C with shaking at 180 rpm. Cultures were
242 centrifuged for 5 min at 6000 rpm and washed with sterile ultrapure water twice after
243 reaching logarithmic phase. The washed bacteria with OD_{600nm} of 0.01 were
244 inoculated into carbon fixing media with 100 µg/ml ampicillin, Each sample had three
245 replicates with 5 ml liquid media in the tubes. OD_{600nm} was determined after
246 incubation at 30 °C for 12 h (Supplementary Fig. S4).

247

248 **Construction of a *S. cerevisiae*/*E. coli* Shuttle Vector.** pOG1 in *E. coli* was
249 propagated overnight in LB medium with 100 µg/ml ampicillin at 37 °C at 220 rpm.
250 *OG1* gene was amplified with AlphaOG1 primer
251 5'-TCTCTCGAGAAAAGAATGACCCCTAACAGGG and 3OG1 primer

252 5'-GATCTAGACTATGGTGAGCTCAGG. The PCR mixture (50 μ l) consisted of 5 μ l
 253 of 10x PCR Buffer (Mg^{2+} plus), 1 μ l of template, 1.5 μ l of 10 mM dNTP mix, 1 μ l of
 254 10 mM upstream primer, 1 μ l of 10 mM downstream primer, 0.3 μ l of Taq enzyme (5
 255 U/ μ l), and sterilized distilled water up to 50 μ L. PCR conditions were as follows: 2
 256 min initial denaturation at 94 $^{\circ}$ C; then 35 cycles of 95 $^{\circ}$ C for 20 s, 55 $^{\circ}$ C for 45 s, 72
 257 $^{\circ}$ C for 90 s; and finally 60 s at 72 $^{\circ}$ C. PCR products were purified by Gel&PCR
 258 purification kit (Promega, Madison, WI, USA). The PCR amplicons and
 259 pYES2/CT/ α -factor (Changsha Yingrun Biotechnology Co., China) were digested
 260 with *Xho* I and *Xba* I for 4 hours at 37 $^{\circ}$ C, followed by inactivation at 70 $^{\circ}$ C for 10
 261 minutes and ligation at 16 $^{\circ}$ C. The ligation products were purified via Gel&PCR
 262 purification kit, and electroporated into *E. coli* DH5 α , which was then plated on a LB
 263 agar plate supplemented with 100 μ g/ml ampicillin and incubated overnight at 37 $^{\circ}$ C.
 264 The positive colonies were verified by PCR followed by sequencing. The resultant
 265 plasmid was designated pYES2OG1.

266

267 **Directed evolution.** pOG1 clone in *E. coli* was propagated overnight in LB medium
 268 with 100 μ g/ml ampicillin at 37 $^{\circ}$ C at 220 rpm. *OG1* gene was amplified with OG1F0
 269 primers 5'-TCGAATTCATGACCCCTAACAGGG and OG1R0 primers
 270 3'-AGTGGATCCCCCGGGCTGCAGCTATGGTGAGCTCAGG. Instant Error-prone
 271 PCR Kit was a product of Beijing Tianenze Gene Technology Co., Ltd. (Beijing,
 272 China)¹⁷. The Error-Prone PCR mixture consisted of 5 μ l of 10x Error-Prone PCR
 273 mix, 5 μ l of 10x Error-Prone PCR proprietary dNTP, 5 μ l of 5mM $MnCl_2$, 1 μ l of
 274 template, 0.5 μ l of 10 μ M upstream primer and downstream primer each, 0.6 μ l of Taq
 275 DNA polymerase (5 U/ μ l), and sterilized distilled water up to 50 μ l. Error-Prone PCR
 276 conditions were as follows: 40 cycles of 98 $^{\circ}$ for 10 s, 55 $^{\circ}$ C for 55 s, 72 $^{\circ}$ C for 60 s.
 277 PCR products were purified by Gel&PCR purification kit. Both PCR amplicons and
 278 pSK- vector were double digested with *Eco*R I and *Bam*H I at 37 $^{\circ}$ C for 4 h, followed
 279 by inactivation at 80 $^{\circ}$ C for 15 minutes and ligation at 16 $^{\circ}$ C. The ligation products
 280 were purified and recovered by Gel&PCR purification kit, and electroporated into *E.*

281 *coli* DH5α cells, followed by plating on a LB agar plate supplemented with 100
 282 μg/mL ampicillin and incubating overnight at 37 °C. The positive colonies were
 283 verified by colony PCR followed by sequencing. The resultant plasmid was named
 284 pOGDR1, pOGDR2, pOGDR3, pOGDR4, pOGDR5, pOGDR6, pOGDR7,
 285 pOGDR10. After an initial one-sample screening via isotopic experiment, clones with
 286 higher carbon fixing activities were further investigated in subsequent experiments.

287

288 **Real-time fluorescent quantitative PCR.** The experiment was contracted with
 289 FitGene BioTechnology Co., LTD (Guangzhou, China)¹⁸. Briefly, an aliquot of the six
 290 samples were collected at the end of a 4-day carbon fixing experiment. RNA was
 291 extracted using RNAPrep Pure Cell/Bacteria Kit according to the instructions of the
 292 manufacturer (TIANGEN Biotech Co., Ltd, Beijing, China). The RNA purity was
 293 determined by UV spectrophotometer SMA4000 (Merinton, China). The RNA was
 294 added to the gDNA adsorption column and centrifuged at 10000 g for 1 min at room
 295 temperature for removal of genomic DNA. The RNA was thermal denatured at 65 °C,
 296 and immediately cooled on ice for 2 min, and used as template for reverse
 297 transcription. Synthesis of cDNA was performed in 10 μL reactions per
 298 manufacturer's instructions. After the reaction, the cDNA was diluted 5 times with
 299 sterilized deionized water and kept at -20 °C. Real-time fluorescent quantitative PCR
 300 was then conducted. 20 μl PCR reactions containing template cDNA, 1x Bestar®
 301 SybrGreen qPCR Mastermix, 1x ROX, 0.2 μM of each primer, and sterilized distilled
 302 water were performed in an ABI7500 thermal cycler (Life Technologies, Carlsbad,
 303 CA, USA). The cycling conditions comprised 2 min polymerase activation at 95 °C,
 304 45 cycles at 95 °C for 10 seconds, 60 °C for 34 s (Fluorescence signal acquisition) and
 305 72 °C for 30 s. In the end of the cycle, melting curve from 60 °C to 98 °C was
 306 obtained. The specificity of the amplifications was verified by melt curve analysis.
 307 Gene expression levels were normalized (ΔΔCt analysis) to 16S rRNA gene
 308 expression levels from the same sample. Gene expression from relative real-time
 309 quantitative PCR experiment was determined using $2^{-\Delta\Delta C_T}$ method. All data are

310 expressed as the means $\pm$ standard deviation.

311

312 **Treatments with carbonic anhydrase inhibitor acetazolamide on CO₂ capture.**

313 pSK- clone and pOG1 clone in *E. coli* MG1655 were each divided into two groups
314 according to the presence (+) or absence (-) of 50 μ g/ml acetazolamide (Sigma,
315 Missouri, USA) in the media. Each group had three duplicates. CO₂ capture experiment
316 was conducted as aforementioned.

317

318 **Statistical analyses.** Statistical analyses were performed using SPSS 22.0. The
319 experiments were evaluated using the Univariate General Linear Model or
320 independent samples-T test when data were normal distributed or approximately
321 normal distributed after examining with Shapiro-Wilk tests. The alpha level for all
322 tests was 0.05. Games-Howell post hoc tests were conducted when equal variance was
323 not assumed.

324 The OG sequence in the original positive clone has been deposited in GenBank
325 with the accession number of KX255659. *OGI* sequence is identical to a GenBank
326 sequence with the accession number NC_012920, ranging from 9251 to 9655 bp. Its
327 encoded protein OK/SW-CL.16 was previously deposited in GenBank with the
328 accession number of BAB93516.

329

330 **Data Availability.** All data generated or analyzed during this study are included in
331 this published article and its Supplementary Information file.

332

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- 383

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400

401 **Figure 1.** $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for CO₂ capture experiments

402 **A,B,C.** Effects of ATP usage on $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, OD600nm post-carbon capture. Orders of the rest of
403 the panels are likewise. **D.** $\delta^{13}\text{C}$ with *S. cerevisiae* clones. **G.** $\delta^{13}\text{C}$ by clones obtained via directed
404 evolution. From A to I, all were propagated in media at pH 8.0. **J.** $\delta^{13}\text{C}$ from clones propagated in
405 media at pH 7.0. Statistical evaluations for $\delta^{13}\text{C}$ are as follows (univariate General Linear Model,
406 two tailed, and $n = 3$ unless specified): **A.** 0.041 (pOG1: ATP vs absence of ATP). **D.** 0.001
407 (pYES2OG1 vs pYES2/CT α -Factor; $n=5$). **G.** 0.012 (pOG1 vs pOGDR1; Games-Howell post
408 hoc tests). **J.** 0.032 (pOG1 vs pSK-; Games-Howell post hoc tests), 0.047 (pSK- vs pOGDR1;
409 Games-Howell post hoc tests). The data were presented as average values with one standard
410 deviation.

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415 **Figure 2.** A, B, C. Effects of acetazolamide usage on $\delta^{13}\text{C}$, δN^{15} , OD600nm post-carbon capture.

416 Treatments with carbonic anhydrase inhibitor acetazolamide (n=3). (+): Presence of 50 $\mu\text{g/ml}$

417 acetazolamide in the media. (-): No acetazolamide in the media. The data were presented as

418 average values with one standard deviation.

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422 **Figure 3.** *OG1* gene expression in *E. coli* clones post- CO_2 capture.

423 **A.** $\delta^{13}\text{C}$. **B.** δN^{15} . **C.** $\text{OD}_{600\text{nm}}$ post- CO_2 capture. **D.** Relative gene expression of *OG1* in the ratio
424 of gene expression in pOG1/pSK- clones in *E. coli* MG1655 cells. Statistical evaluations for $\delta^{13}\text{C}$
425 are as follows (two tailed, and $n=3$): **A.** 0.015 (pOG1 vs pSK-; univariate General Linear
426 Model). **D.** 0.008 (pOG1 vs pSK-, t test). The data were presented as average values with one
427 standard deviation.

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430 **Table 1.** Mutants from directed evolution

Name	GenBank Accession no.	Site in the OG1 gene	DNA Base mutation	Amino acid substitution
pOGDR1	MF968946	322	C>A	P108T
pOGDR2	MF968952	237	T>C	Nonsense mutation
pOGDR3	MF968947	113	A>G	N38S
pOGDR4	MF968948	289	A>G	I97V
pOGDR5	MF968949	389,96	A>C, T>C	H130P
pOGDR6	MF968950	389	A>C	H130P
pOGDR7*	JX462739	269	A>G	Q90R
pOGDR10	MF968951	169	C>T	H57Y

431 *OGDR7 shares homology with *Homo sapiens isolate P75 mitochondrion*

432 (GenBank Accession no.: JX462739)